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Local Conformational Preferences of Peptides Near Attached Cations: Structure Determination by First-Principles Theory and IR-Spectroscopy
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In the transition from secondary to tertiary structure in peptides and proteins, turns take a special role. They are the hinges that arrange the periodic secondary structure elements (helices and strands) to the native fold. It is a known effect that Li^+ alters peptide backbone structure,[1] and we investigate this effect on the structure and dynamics of turns for model peptides Ac-Ala-Ala-Pro-Ala-NMe (AAPA) and Ac-Ala-Asp-Pro-Ala-NMe (ADPA) by theoretical conformational predictions and experimental vibrational spectroscopy. On theory side, accurate conformational predictions can not succeed without a trustworthy description of the potential energy surface, but standard force fields lack this detailed accuracy for the ion-peptide systems investigated. We show that accurate predictions can be achieved by a first-principles approach (van der Waals corrected density functional theory (DFT) in the PBE generalized gradient approximation[2]), and verify all our predictions by comparison to infrared spectroscopy in the same clean-room environment (spectra obtained *in vacuo*, using the FELIX free-electron laser facility).

We predict canonical turn conformations for AAPA and ADPA alone. Li^+ and Na^+ , by adsorbing to C=O groups, induce unusual backbone conformations and prevent H bond formation. We show that accounting for finite-temperature free energy contributions (harmonic approximation) is essential for a consistent comparison between theoretically predicted conformers and experimental spectra. The comparison suggests that multiple conformers coexist at finite temperature, based on theoretically derived spectra including anharmonic effects for individual conformers by *ab initio* Born-Oppenheimer molecular dynamics (MD) simulations. Intriguingly, some of the predicted low-energy conformers contribute less than others to the observed spectra. The same MD simulations give insights into backbone motion patterns like peptide bond crankshaft rotation.

[1] Seebach et al. Modern Synthetic Methods 7, 1 (1995).

[2] Tkatchenko, Scheffler, Phys. Rev. Lett. 102, 073005 (2009).

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Structural Analysis of Human Apolipoprotein E3 by Fluorescence Spectroscopy, and Hydrogen/Deuterium Exchange Coupled to Mass Spectrometry

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Apolipoprotein E3 (apoE3) is an important anti-atherogenic protein that helps maintain cholesterol levels in the brain and plasma. It is responsible for binding and cellular uptake of plasma lipoproteins via the low-density lipoprotein receptor family of proteins. It is a highly alpha-helical protein that can exist in lipid-free and lipid-bound states. ApoE3 is composed of two domains in lipid-free state: an N-terminal (NT) domain folded into a 4-helix bundle and a C-terminal (CT) domain that mediates apoE3 oligomerization via inter-molecular helix-helix interactions. The objective of this study is to understand the conformational organization of lipid-free apoE in its oligomeric state. We employed chemical-induced denaturation coupled to fluorescence spectroscopy of apoE bearing environmentally sensitive fluorescent probes monitoring different helices in the two domains, to obtain information regarding tertiary conformation. In a complementary approach, we also used hydrogen/deuterium exchange coupled to mass spectrometry (HDX-MS) to understand amide-backbone structural dynamics, solvent accessibility, and helical contours of apoE3. Fluorescence intensity and polarization studies indicate that the unfolding is likely initiated at the C-terminal end of the protein, the CT domain unfolds prior to NT domain, and that the NT domain forms a highly stable helix bundle. HDX/MS analysis revealed that the amide backbone of the NT domain underwent limited exchange, with the exception of the first 14 residues at the N-terminal end, and those linking helices 2 and 3 (79-93), confirming the presence of a tight helix bundle. In contrast, the CT domain revealed significantly higher HDX rates. Our studies suggest that the two domains of apoE may undergo independent conformational reorganization, a concept that bears significant relevance in terms of apoE interaction with lipids and lipoproteins. NIH-HL096365 and TRDRP 17RT-0165.

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Biophysical and Biochemical Characterization of Acrolein-Modified Apolipoprotein E

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Apolipoprotein E (apoE), an anti-atherogenic apolipoprotein, plays a significant role in the metabolism of lipoproteins. It lowers plasma lipid levels by acting as a ligand for low-density lipoprotein receptors (LDLr). ApoE mediates this function via essential lysine residues that interact with the LDLr. Our preliminary study shows that rats exposed to environmental tobacco smoke displayed oxidative modification of apoE and dissociation of lipoprotein-bound apoE. The objective of this project is to study the effect of oxidative stress (specifically acrolein) mediated *in vitro* modification on the structure and function of recombinant rat apoE. SDS-PAGE and RP-HPLC confirmed that the protein was purified to homogeneity with no signs of degradation. Acrolein modification of apoE was confirmed by Western blot analysis. Circular dichroism and fluorescence spectroscopy revealed that the secondary and tertiary structures of acrolein-modified apoE were affected with significant difference in the overall fold of the modified protein. Modified apoE also demonstrated a decrease in binding affinity for heparin and lipid binding ability. Lastly, the LDLr binding ability of acrolein-modified apoE was significantly impaired. Overall, we conclude that acrolein disrupts the structural and functional integrity of apoE, which is likely to affect its role in maintaining plasma cholesterol homeostasis. Our data provide a molecular basis for the potential role of oxidative stress mediated Modification of apoE in altering lipoprotein metabolism, with direct implications in cardiovascular disease.

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Small Molecules CK-666 and CK-869 Block an Activating Conformational Change to Inhibit Arp2/3 Complex

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Arp2/3 complex is a seven subunit assembly that nucleates actin filaments from the sides of pre-existing filaments, creating branched actin networks. Recently reported small molecule inhibitors of Arp2/3 complex, CK-666 and CK-869, block its nucleation activity. While these inhibitors have the potential to become powerful tools to investigate actin remodeling *in vivo*, it is currently not known how they inhibit Arp2/3 complex. To determine the mechanism of these inhibitors, we conducted biochemical/biophysical analysis and solved the crystal structures of CK-666 and CK-869 bound to Arp2/3 complex. Despite the fact that CK-869 and CK-666 bind to distinct sites on the complex, both compounds function by blocking an activating conformational change stimulated by dimeric VCA and two actin monomers. Chemical crosslinking, analytical ultracentrifugation, and small angle x-ray scattering demonstrate that this conformational change moves Arp2 and Arp3 together to adopt the short pitch conformation, mimicking two consecutive actin subunits in an actin filament and is hypothesized to form the nucleus for the daughter filament. Analysis of the crystal structures shows that CK-666 binds to a pocket between Arp3 and Arp2 formed only in the inactive conformation, stabilizing the inactive state. In contrast, binding of CK-869 locks a surface loop in Arp3 in a position, destabilizing the short pitch conformation. Neither inhibitor actively dissociates preformed branches, nor significantly affects binding of ATP or nucleation promoting factors to the complex or binding of Arp2/3 complex to the sides or ends of actin filaments. Therefore, CK-666 and CK-869 block a late step in the nucleation pathway by preventing a large-scale conformational change. These results have important implications for understanding the branching nucleation mechanism and will be critical in interpretation of the effects of the inhibitors on Arp2/3 complex activity *in vivo*.

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HIV-1 Reverse Transcriptase Monomers Adopt Multiple Conformations in Solution

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HIV-1 reverse transcriptase (RT) is a key enzyme in HIV infection and an important therapeutic target. The enzyme is an asymmetric heterodimer of p66 and p51 subunits. Although each subunit has an N-terminal polymerase domain with identical amino acid sequence (440 residues), in the heterodimer the polymerase domain of p66 adopts an open conformation while that of p51 is in a closed conformation. The p66 and p51 monomers are folded proteins of